Supporting Information

Dendritic Chelating Agents 1. Cu(II) Binding to Ethylene Diamine Core Poly(amidoamine) Dendrimers in Aqueous Solutions

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By

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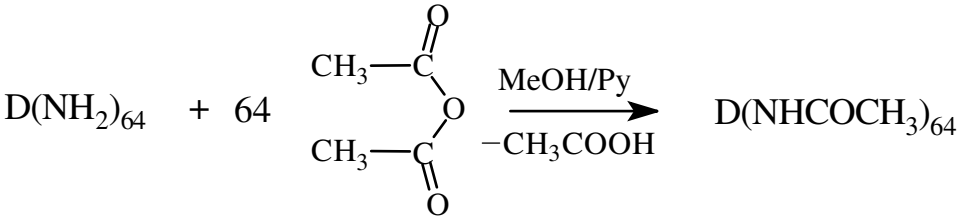
1. Dendrimer Synthesis

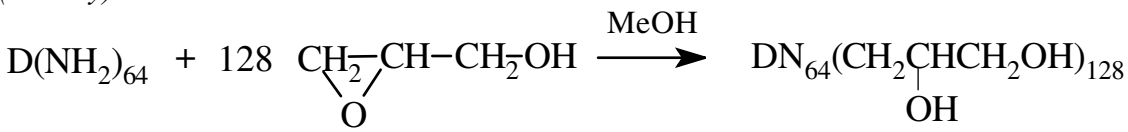
Generation 4 EDA core PAMAM dendrimer was purchased from Dendritech (Midland, MI) as methanol solution (26 wt%). Acetic anhydride, pyridine, succinic anhydride, glycidol and all the remaining chemicals/solvents were purchased from Aldrich. Milli-Q deionized water (with resistivity >18 MΩcm) was used in all of the experiments. Regenerated cellulose dialysis membranes were purchased from Fisher Scientific.

G4 EDA core PAMAM Dendrimer with Acetamide ($NHCOCH_3$) Terminal Groups (G4-Ac)



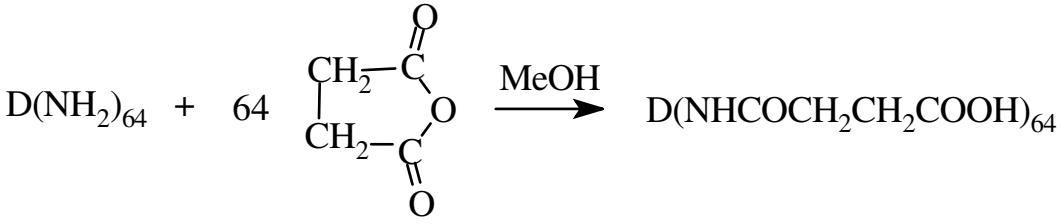
4.1 mL of pyridine was added to 11.56 g of a solution of EDA core G4-NH_2 PAMAM dendrimer (3.0056 g) in methanol. The resulting solution was diluted to 40 mL by addition of methanol in a 250-ml round-bottom flask. A solution of 2.6 g acetic anhydride in methanol was then added drop wise to the dendrimer/pyridine mixture under continuous stirring. The resulting solution was equlibrated for 24 hours. The methanol was then removed from the reaction mixture on a rotary evaporator. The oily crude product was diluted and dialyzed with deionized water (6 X 4 liters) for three days to remove the excess reactants and byproducts. The final product was removed from the aqueous retentate, dissolved in deionized water and lyophilized. The yield (3.15g) was estimated at 88.2%.

G4 EDA core PAMAM Dendrimer with glycidol ($NHCH_2CH(OH)CH_2OH$) Terminal Groups (G4-Gly)



1.9 g of glycidol (in methanol solution) was added drop wise to 11.54 g of a solution of EDA core G4-NH_2 PAMAM dendrimer (3.0004 g) in methanol in a 250-mL round bottom flask under continuous stirring. The resulting mixture was equilibrated for 24 hours and the methanol was removed on a rotary evaporator. The oily crude product was diluted and dialyzed with deionized water (6 X 4 liters) in three days to remove the excess reactants and byproducts. The aqueous retentate was dissolved in deionized water and lyophilized. The yield (3.92 g) was estimated at 98.0%.

G4EDA core PAMAM Dendrimer with Succinamic Acid ($(NHCOCH_2CH_2COOH)$ Terminal Groups (G4-Sac)



3.67 g of dried EDA core G4-NH_2 PAMAM dendrimer was added to 50 mL of dimethyl siloxane (DMSO) in a 250-mL round bottom flask. 50 mL of DMSO solution containing 3.1 g of succinic anhydride was added to the dendrimer/DMSO mixture under continuous stirring for 24 hours. The reaction mixture was dialyzed against water to remove the excess succinic anhydride and DMSO. The aqueous retentate was filtered and then lyophilized. The yield (4.66 g) was estimated at 87.5%.

2. Dendrimer Characterization Procedures

The dendrimers evaluated in this study were characterized by $^{1}H/^{13}C$ NMR spectroscopy, HPLC, capillary electrophoresis (CE), polyacryl amide gel electrophoresis (PAGE), size exclusion chromatography (SEC) and matrix-assisted laser desorption (MALDI) time of flight (TOF) mass spectrometry.

NMR and HPLC Characterization. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 500 spectrometer. ^{1}H-NMR spectra were collected at 500.1 MHz using $CHCl_3$ (7.259 ppm) as internal reference and ^{13}C NMR spectra were obtained at 125.8 MHz using $CDCl_3$ (77.23 ppm) as internal reference. A Beckman Gold System HPLC system consisting of a 507 Autosampler, a Gold 166 detector and a Gold 126 solvent module was used to characterize the EDA core PAMAM dendrimers. A reverse-phase ion-pairing HPLC method was employed in all analyses. This method utilizes trifluoroacetic acid (TFA) as an ion-pairing reagent and acetonitrile as an organic modifier with wavelength of detection at 210 nm. A linear gradient (40% in 60 minutes) was applied starting from 0.1% aqueous TFA and gradually modifying with 0.07% TFA/CH_3CN solution.

Capillary Electrophoresis (CE). An CE instrument from Agilent Technologies (Waldbronn, Germany) was used in this study. Quartz capillaries were purchased from Polymicro Technologies (Phoenix, AZ). The capillary temperature was maintained at 40 °C, and the separation voltage was kept at 20 kV in all cases. On-capillary UV diode-array detection system was used. The detector was operated at wavelengths of 200 nm, 210nm, 250nm and 300nm. Samples were introduced by hydrodynamic injection at a pressure of 50 mbar. Silanized capillaries (internal diameter of 100 μm with total length 78.5 cm and effective length 70 cm) were employed to characterize the EDA core Gx-NH_2, G4-Gly and G4-Ac PAMAM dendrimers. Prior to use, each silanized capillary was rinsed with a 0.2 M H_3PO_4 solution and washed with deionized water followed by a rinse with the running buffer. Before sample injection, each capillary was also rinsed with a 0.2 M H_3PO_4 solution in deionized water followed by a rinse with the running buffer. A 50 mM phosphate buffer (pH = 2.5) was employed in all experiments. A 0.03 mg/mL 2,3-diaminopyridine (2, 3-DAP) solution was used as an internal standard.

Bare silica capillaries (internal diameter of 75 μm with total length 48.5 cm and effective length 40 cm) were used in the characterization of the EDA core G4-Sac PAMAM dendrimer. Prior to the CE analyses, each capillary was rinsed with a 1 M NaOH solution in deionized water and the running buffer. The capillary temperature was kept at 20 °C. Before each injection, the capillary was also rinsed with a 1 M NaOH solution in deionized water followed by a rinse with the running buffer. A 20 mM borate solution (pH = 8.3) was used as the running buffer. Aliquots of G4-Sac PAMAM dendrimers were dissolved in the buffer solution and their pH were adjusted to 8.3 to final concentrations of 1 mg/mL. Mesityl oxide (0.5 mg/mL buffer) was employed as neutral marker.

Polyacrylamide Gel Electrophoresis (PAGE). Characterization of PAMAM dendrimers by PAGE was carried out on a Micrograd vertical electrophoresis system (Gradipore, Sydney, Australia). Precast 4-20% gradient express gels for PAGE were obtained from ISC BioExpress (Kaysville, UT). A commercial power supply (Model 500/200; BioRad, Hercules, CA) was used. Electrophoresis experiments were performed on 10 × 8 cm gels in a vertical electrophoresis unit (Model Protean I; BioRad). Tris-Glycine (TG) buffer (pH = 8.3) was purchased from Invitrogen (Carlsbad, CA) and diluted by 10 times to prepare the running buffer. PAGE separations typically required 50 min at 200 V. Reverse polarity was used for the analysis of the EDA core Gx-NH_2, G4-Gly and G4-Ac PAMAM dendrimers. Into every sample, a 2 μL of a sample solution containing 1 μL of dendrimer solution (1mg/mL) and 1 μL of a methylene blue sucrose dye (50% sucrose and 1% methylene blue) was injected. For the EDA core G4-Sac PAMAM dendrimer, 1 μL of a dendrimer solution (1mg/mL) and 1 μL bromophenol blue sucrose dye (50% sucrose, 1% bromophenol blue) were injected. Developed gels were stained overnight with 0.025% Comassie Blue R-250 in 40% methanol and 7% acetic acid aqueous solution. The gels were destained with 7% (v/v) acetic acid and 5% (v/v) methanol in water.

Size Exclusion Chromatography (SEC). SEC experiments were performed on an Alliance Waters 2690 instrument equipped with a 2487 Waters dual wavelength UV detector, a Wyatt Dawn DSP laser, an Optilab DSP refractometer (Wyatt Technology Corporation). TosoHaas TSK-Gel Guard PHW 06762 (7.5x7.5 cm, 12 mm), G 2000 PW 05761, G 300 PW 05762, and G 4000 PW

05763, (all 30x7.5 cm, 17 mm) columns were used in the experiments. Isocratic runs were performed at 25 C, at 1 ml/min flow rate using 0.1 M citric acid and 0.0025% sodium azide buffer (pH=2.74) as aluents.

MALDI-TOF Mass Spectrometry. The MALDI-TOF spectra were acquired on a Micromass ToF 2E mass spectrometer using a linear operation mode. 10 mg/mL of β-indoleacrylic acid in acetonitrile/H_2O (v/v=70:30) was used as matrix. Aliquots of 1 mg of dendrimer were dissolved in 1 mL methanol, and then diluted 5 times by methanol to a final concentration of 0.2 mg/mL. Equal volumes of the dendrimer solution and matrix solution were mixed. 1 μL solution of the resulting mixture was then injected into the mass spectrometer. Myoglobin (16951g/mol), Trypsinogen (23976 g/mol) and a 5 picomole protein standard of Cytochrome-C (12359g/mol), were used as external standards.

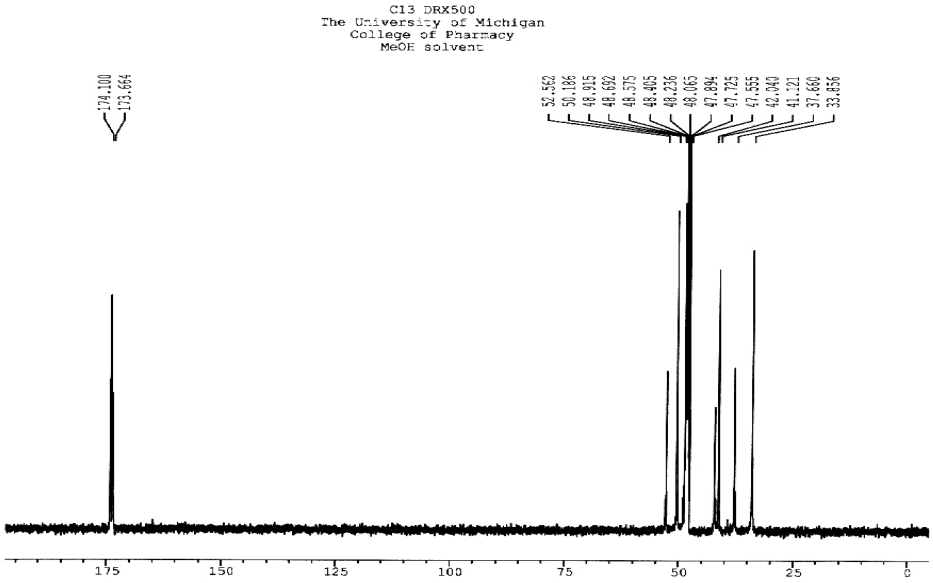
Figures S1, S2 and S3 highlight a typical characterization data set for the dendrimers evaluated in this study as illustrated on the G5-NH_2 EDA core PAMAM dendrimer. This compound contains more impurities and defects than any of the dendrimers evaluated in this study

3. Acid-Base Titration of Aqueous Solutions PAMAM Dendrimers.

The titration experiments were carried out using a computer-controlled QC-Titrate system from Man-Tech Associates (http://www.titrationplus.com/). The pKa of the dendrimer tertiary amine and terminal groups were taken as the values of the solution pH at the inflexion points of the corresponding titration curves (Figures S4 through S9).

Figure S1: Characterization of EDA Core G5 PAMAM Dendrimer with Terminal NH_2 Groups by ^{13}C and ^{1}H NMR.

A. ^{13}C NMR



B. ^{1}H NMR

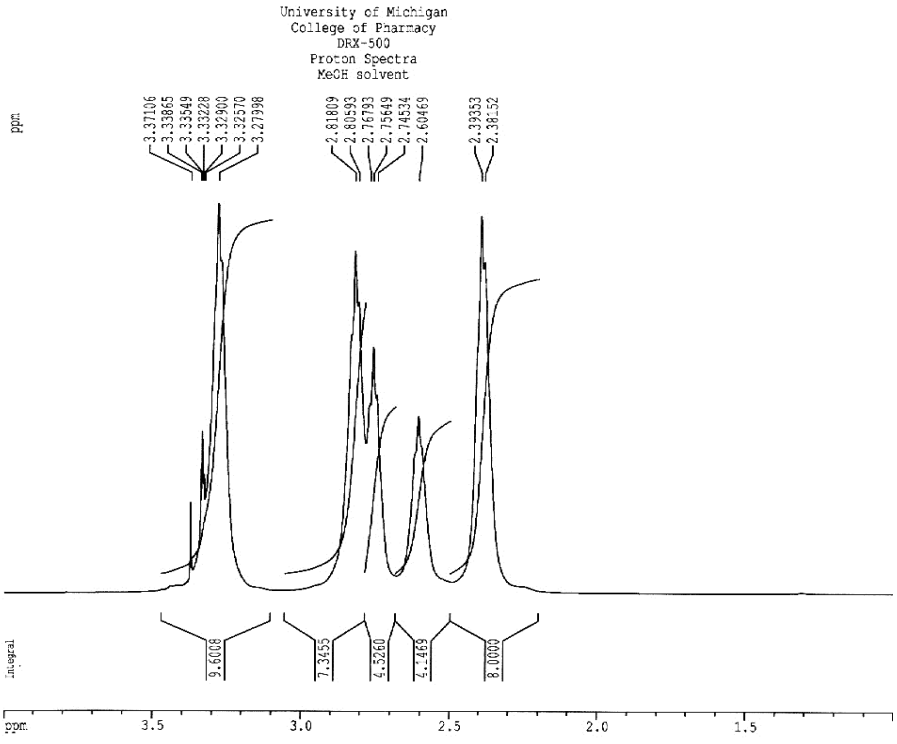
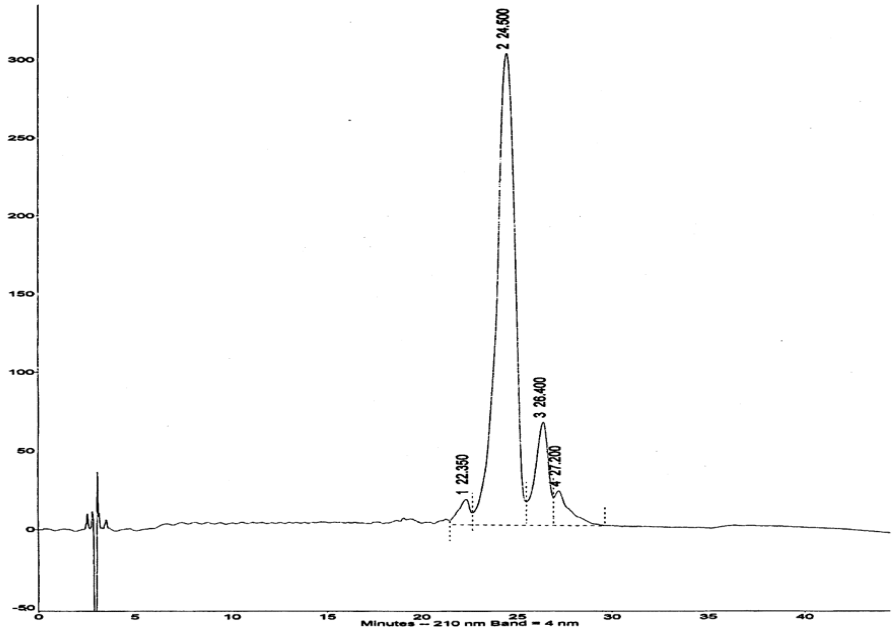


Figure S2: Characterization of EDA Core G5 PAMAM Dendrimer with Terminal NH_2 by HPLC and MALDI MS

A. HPLC



B. MALDI ToF

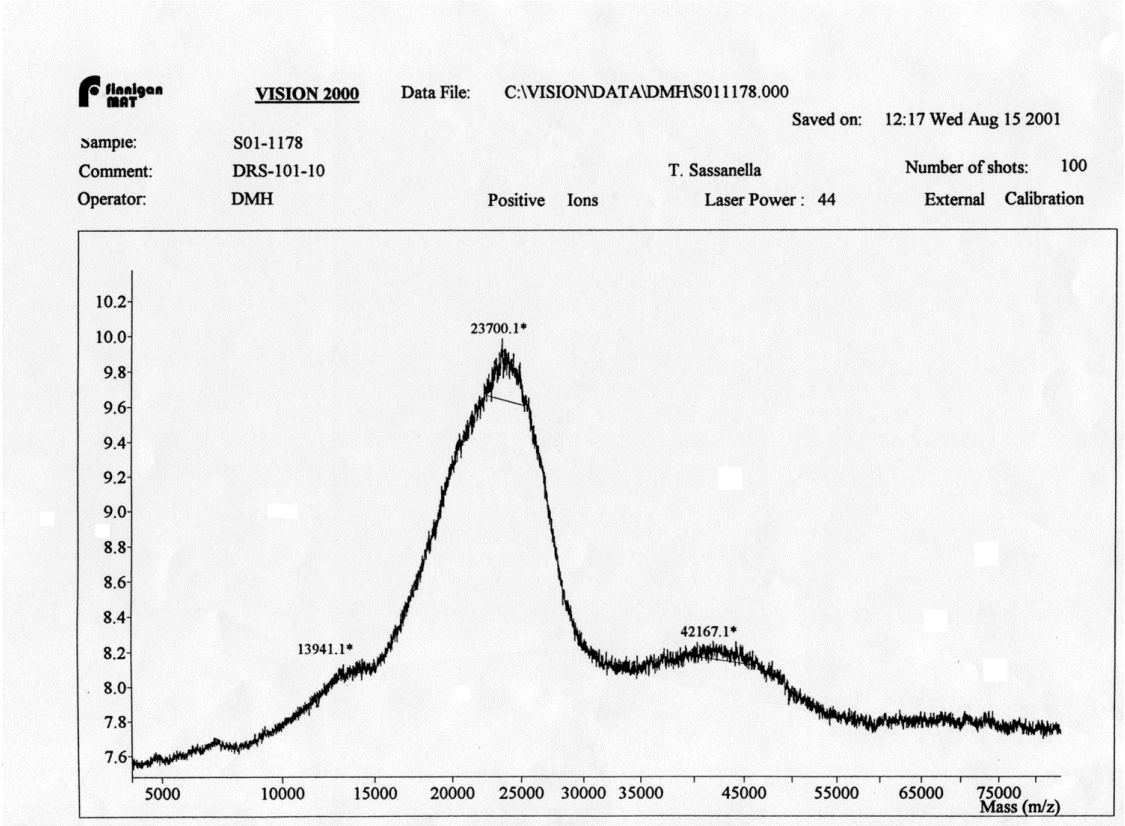


Figure S3: Characterization of EDA Core G5 PAMAM Dendrimer with Terminal NH_2 by Capillary Electrophoresis (CE), Size Exclusion Chromatography (SEC) and Polyacryl Amide Gel Electrophoresis (PAGE)

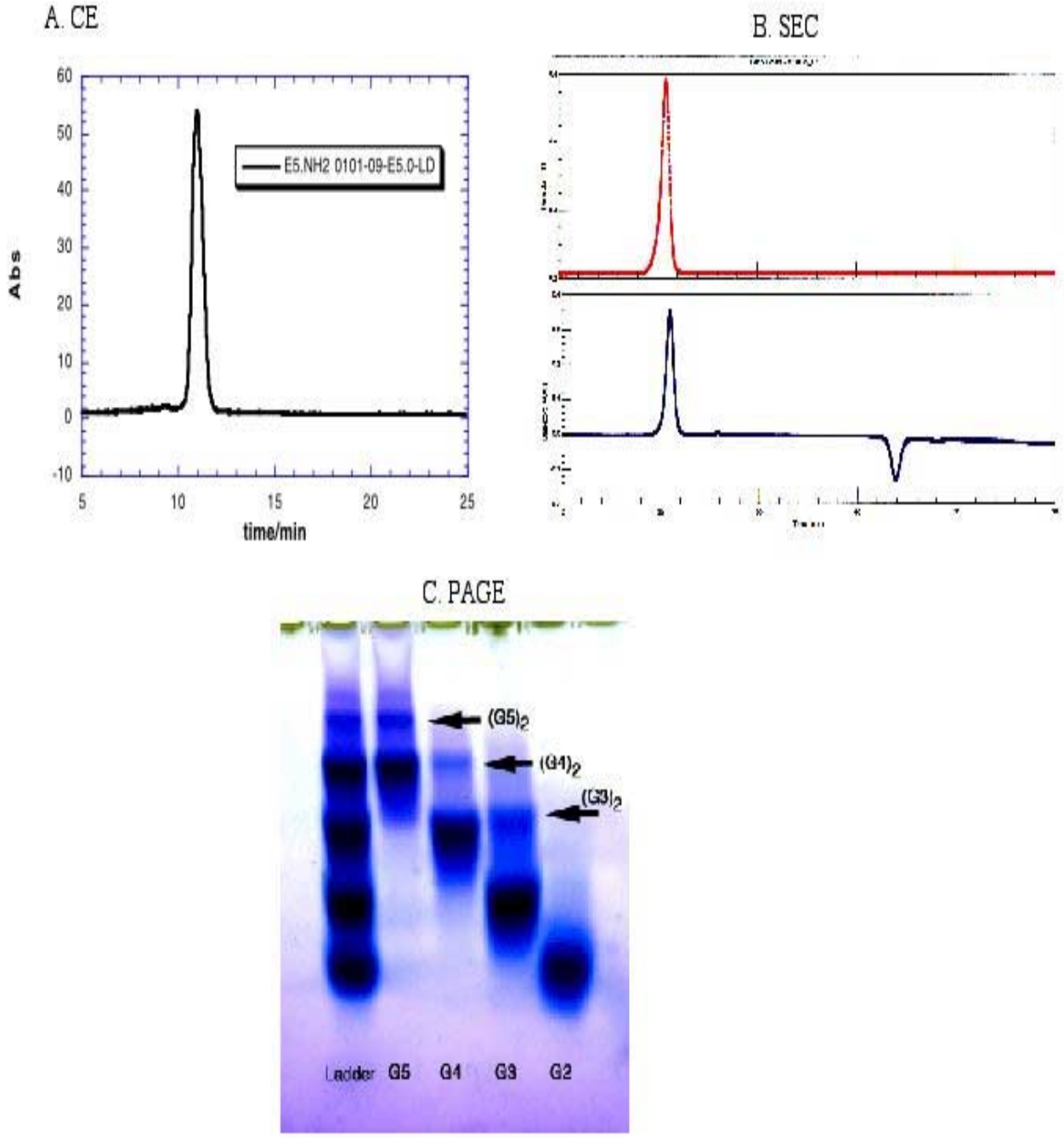


Figure S4: Titration Curve (with First and Derivatives) for EDA Core G3 PAMAM Dendrimer with Terminal NH_2 Groups

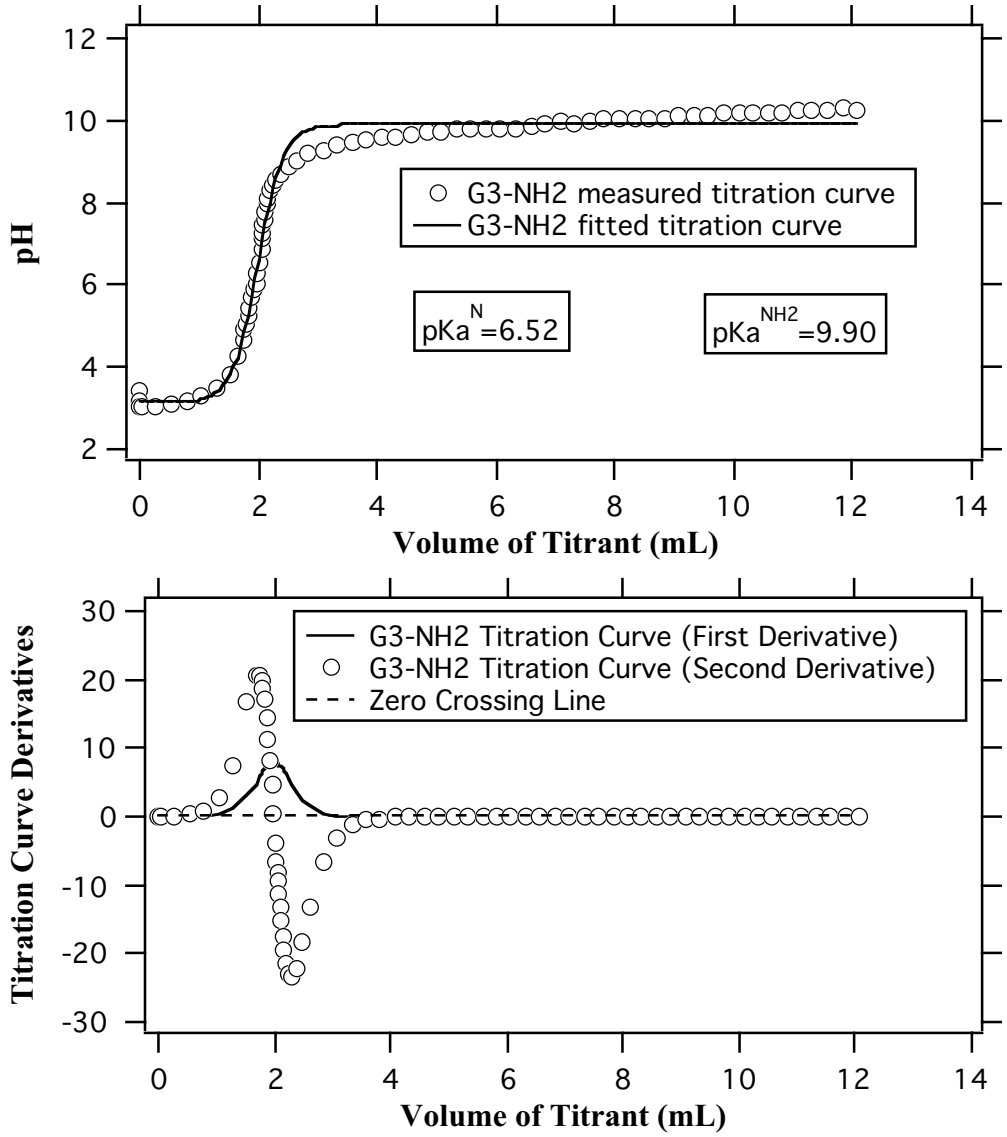


Figure S5: Titration Curve (with First and Derivatives) for EDA Core G4 PAMAM Dendrimer with Terminal NH_2 Groups

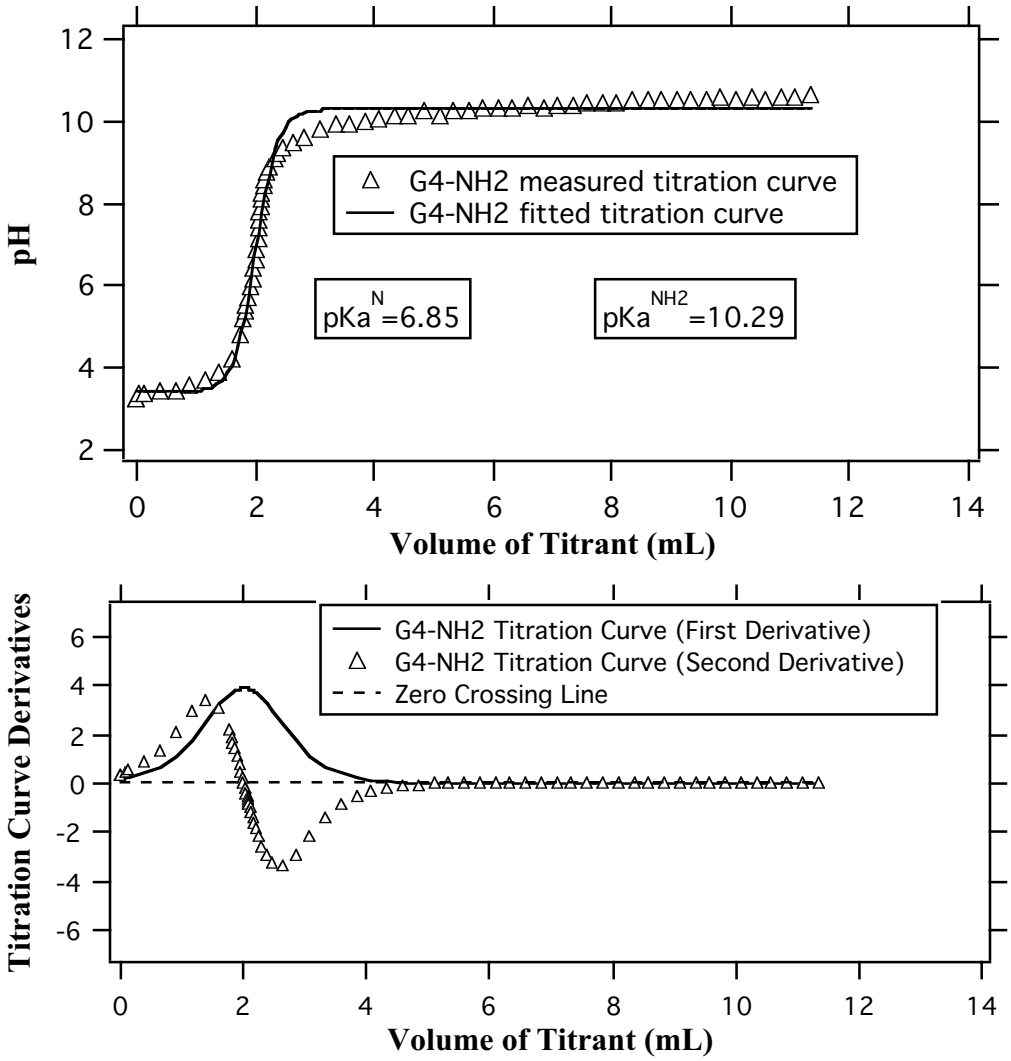


Figure S6: Titration Curve (with First and Second Derivatives) for EDA Core G5 PAMAM Dendrimer with Terminal NH_2 Groups

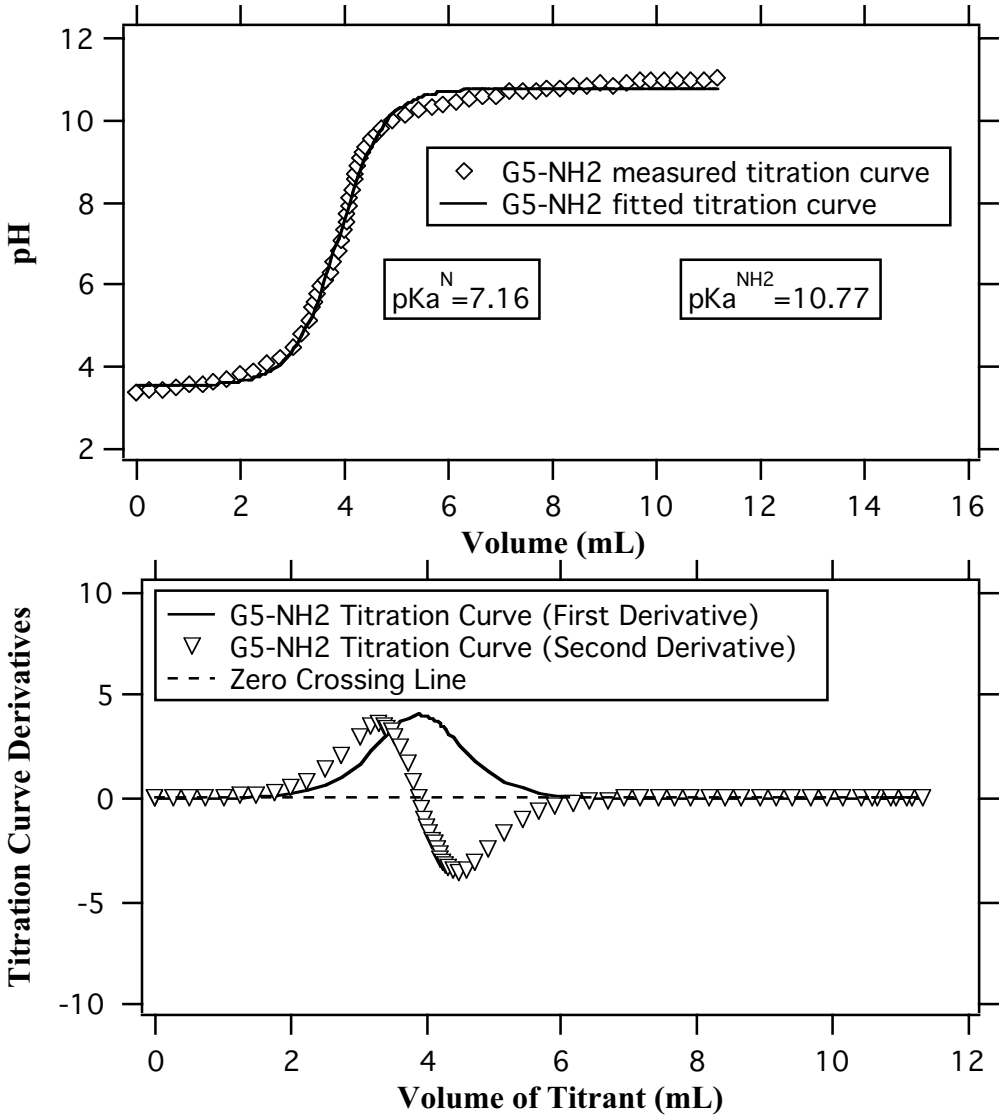


Figure S7: Titration Curve (with First and Second Derivatives) for EDA Core G4 PAMAM Dendrimer with Terminal Succinamic Acid Groups (G4-Sac)

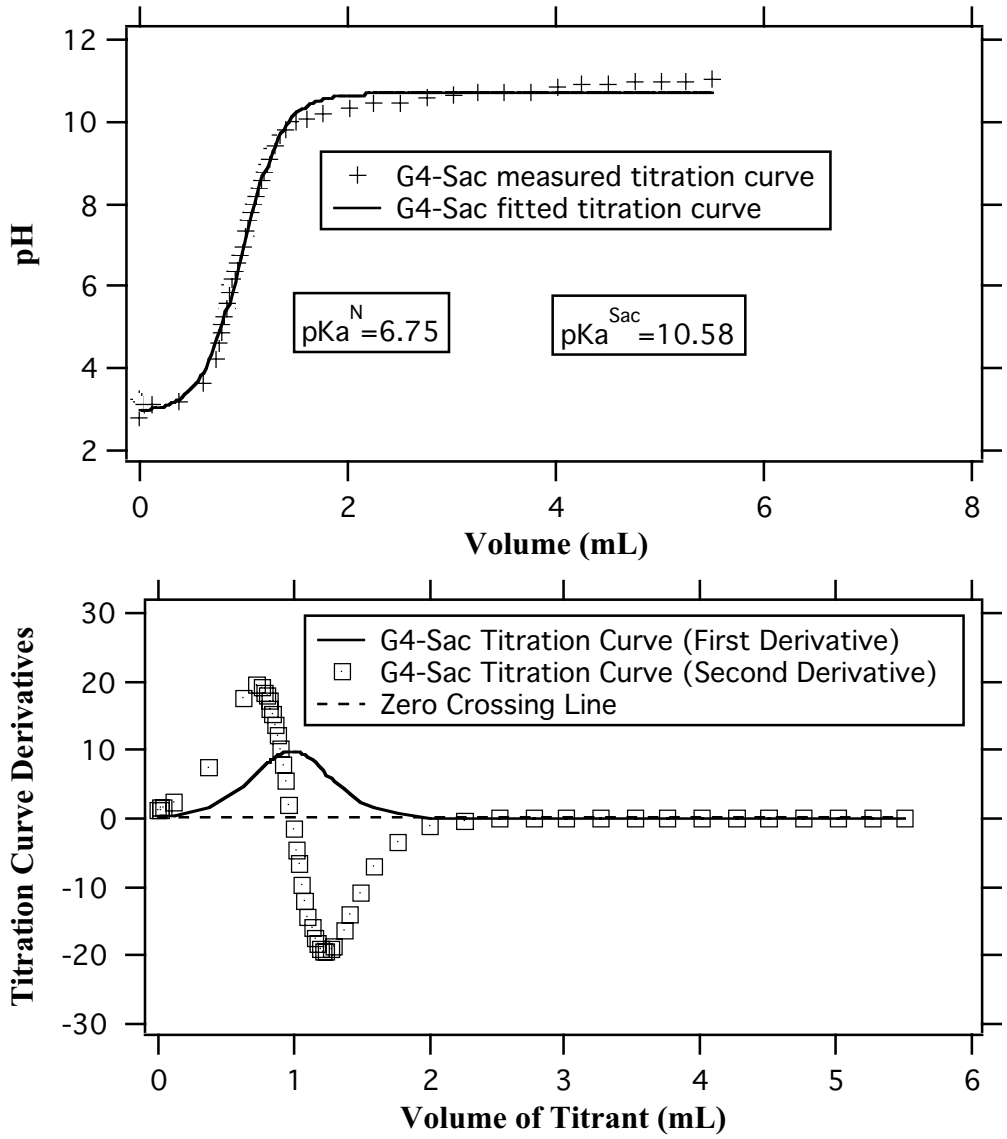


Figure S8: Titration Curve (with First and Second Derivatives) for EDA Core G4 PAMAM Dendrimer with Terminal Glycidol Groups (G4-Gly)

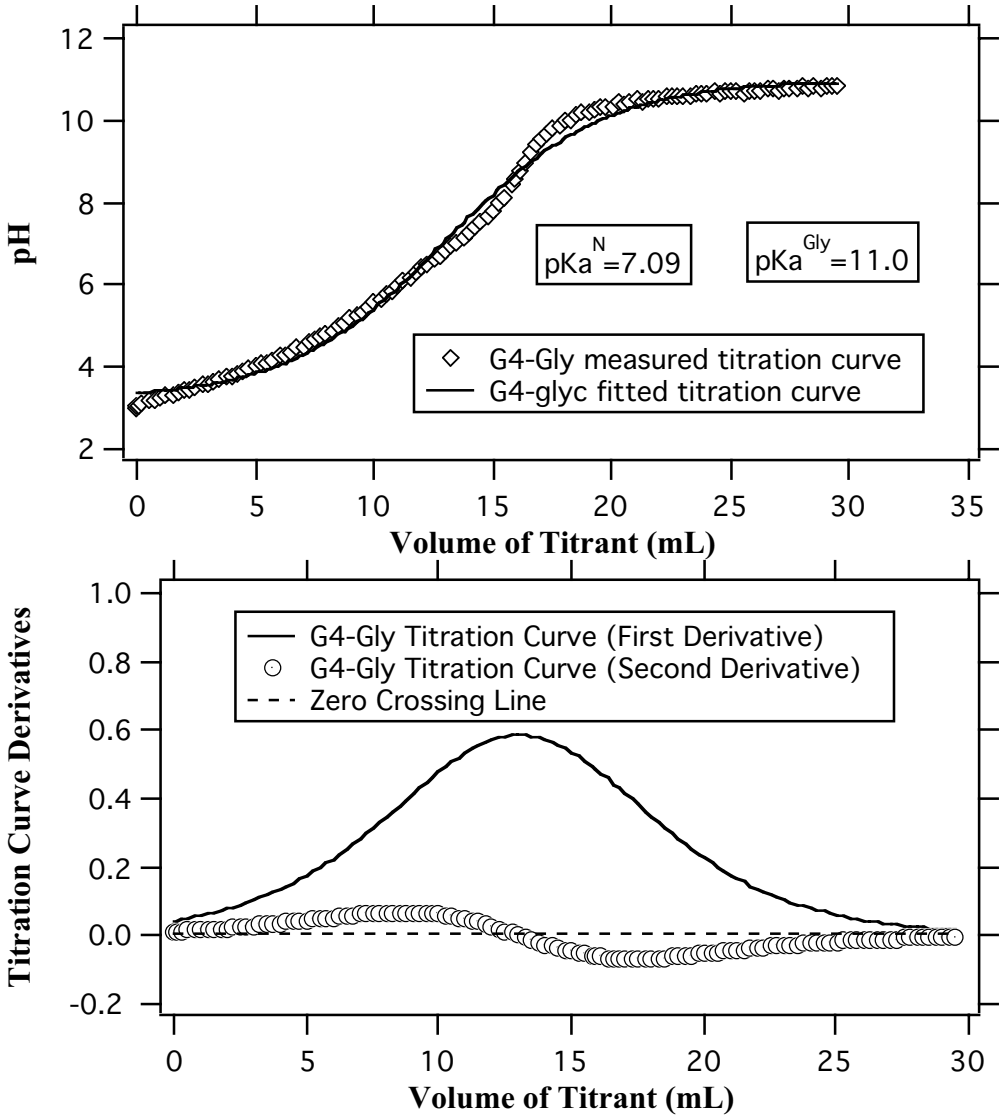


Figure S9: Titration Curve (with First and Second Derivatives) for EDA Core G4 PAMAM Dendrimer with Terminal Acetamide Groups (G4-Ac)